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A WHOLE ANIMAL MODEL FOR *IN VIVO* STUDIES OF THE EFFECTS OF ENVIRONMENTAL (Thermal) STRESS AND VASOACTIVE SUBSTANCES ON PERIPHERAL BLOOD FLOW

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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A whole animal model for *in vivo* studies of the effects of pharmacological vasoactive substances and environmental stress on peripheral blood flow is described. The rat was selected as the basis of the model because its long conical tail lends itself to both venous occlusion plethysmographic measurement of total blood flow and laser Doppler flowmetry for measurement of cutaneous microvascular flow. These two non-invasive measures of blood flow at different levels in the vascular tree provide insight about the location at which substances affect the vasculature. Furthermore, the ability to compare blood flow in the foot pad and tail, i.e., two distinct peripheral vascular beds, allows for assessment of the homogeneity of responses.

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Experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS Publications (NIH) 86-23 (1985).

INTRODUCTION

This report describes the methods and instrumentation used to establish the rat tail and foot as a model for assessing the effects of vasoactive substances and environmental stress (thermal) on peripheral blood flow in a whole animal preparation. Isolated blood vessel preparations are often used to investigate the actions and roles of various neurotransmitters and vasoactive substances on vasculature. Such studies are important and provide a wealth of valuable information about vasoactive substances and the mechanisms of their actions on vascular smooth muscle. They do not and cannot, however, demonstrate what effects these vasoactive substances have in intact organisms. Thus, a whole animal model for *in vivo* studies is essential to fully understanding the role of vasoactive substances in the regulation of peripheral blood flow. *In vivo* studies are essential for establishing the medical relevance and potential use of vasoactive substances in treating peripheral vascular disorders.

The requirements of the whole animal model are the following:

1. peripheral blood flow in macro- and micro-vasculature can be measured and compared in intact whole animals for prolonged periods (> 1 hr);
2. blood flow can be monitored in more than one peripheral vascular bed or extremity to verify the congruity of observed effects;

3. experiments are repeatable in the same animal such that the effect of a given substance at a given dose is verifiable and so that the effects of different substances can be compared in the same preparation;
4. the methods and apparatus must be appropriate for experiments in either alert or anesthetized animals.

Model: Rats (male Long-Evans of 290-310 g) were selected for the model because of their long, conical tail, which is ideal for venous occlusion plethysmographic measures of total blood flow (1), and because it is possible to measure simultaneously superficial cutaneous microvascular flow using laser Doppler flowmetry (2, 3). This combination of non-invasive measures of total and microcirculatory flow provides insight about the level of action of vasoactive substances within the vascular tree. Furthermore, the ability to monitor concurrently microvascular blood flow via laser Doppler flowmetry in another distinct vascular bed, the foot pad, allows for an assessment of homogeneity of responses to vasoactive substances. Venous occlusion plethysmography was selected for measuring total blood flow because it is a sensitive, non-invasive method that provides a quantitative measure of blood flow. The method uses the change in volume of the tail over time, while venous drainage of the tail is temporarily (5 s) occluded, to measure flow (1). Laser Doppler flowmetry is also a non-invasive, state-of-the-art method of measuring qualitative changes in microvascular flow (2). Both methods allow for repeated experiments on the same animal.

Another advantage of selecting the rat as the basis of our model is the many studies that use isolated vessel or isolated organ preparations from rats to assess effects of vasoactive substances. They provide a foundation of information for whole animal studies in this species.

METHODS

Instrumentation and measurements. Parameters measured included superficial cutaneous blood flow in the foot pad and tail, total blood flow in the tail, skin temperature on the foot pad and tail adjacent to the laser Doppler flow probe, ambient temperature, and core temperature. Data collection and instrumentation associated with the venous occlusion plethysmographic measures of total tail blood flow were controlled by an AT compatible computer (Zenith Data Systems, Heath Company), Keithley 575 Measurement and Control System (Keithley Instruments, Inc.), and LabTech Notebook software (Laboratory Technologies Corporation). A fully instrumented rat is depicted in Fig. 1.

Surgical preparation. A cannula (ITTC Life Science, S-26) was implanted in a jugular vein or vena cava, and a polyethylene blind-ended reentrant tube secured adjacent to a carotid artery at least one week prior to experiments. A copper-constantan thermocouple was inserted into the reentrant tube to monitor core temperature. Both tubes were routed subcutaneously to exit through the skin between the shoulders. All surgery was

performed using sterile techniques and while the rat was anesthetized with 50 mg/kg sodium pentobarbital.

Superficial cutaneous microvascular blood flow. Laser Doppler flowmeters (TSI Laserflow, Model BPM 403A) were used to monitor blood flow in the superficial layers of skin in the foot pad and tail (4). Foot pad blood flow was monitored with a right-angle skin probe (No. P-430) secured to the foot pad with double-sided adhesive circles. The position of the probe was determined only when an acceptable minimum baseline blood flow of 20 ml/100ml tissue/min was recorded. Tail skin blood flow was measured with a right-angle implantable prism probe (No. P-434) held to the tail with an in-house designed foam rubber holder. A 3-cm diameter, 4.5-cm long, plexiglas cylinder, filled with a cylinder of foam rubber, was cut in half and hinged on one side like a clamshell. A hole the shape of a rat tail ran lengthwise through the center. The laser Doppler probe was implanted in the bottom half of the foam, such that it protruded slightly into the center hole mid way along the holder. This holder allowed for "fine-tuning" of the probe's position to obtain an acceptable baseline flow level (> 2 ml/ 100 ml tissue/ min) but held it stationary for the duration of an experiment. Tail hair was removed, prior to experiments, using a depilatory (Neet).

Total blood flow in the tail. Venous occlusion plethysmography, a highly sensitive, accurate, quantitative method of measuring total blood flow in an extremity (1), was used to measure total blood flow in the rat tail (5, 6, 7). A

pneumatic venous occlusion cuff, placed at the base of the tail just above the laser Doppler probe holder, was inflated under computer control to 55 mmHg for 5 s at 20-s intervals. A 4.5-cm Whitney (8) mercury-filled silastic strain gauge, positioned ~1 cm distal to the probe holder, was connected to an electronic plethysmograph (6) (Hokansen; model EC-4). The plethysmograph measured changes in the volume of the rat tail by supplying a small voltage through the strain gauge and measuring change in the resistance through the strain gauge as it was stretched. While the venous occlusion cuff temporarily prevents any blood from leaving the tail during the 5-s inflation, the rate of total blood flow is measured as the rate of change in volume of the tail (at the level of the strain gauge). The signal from the plethysmograph was sampled at 1-s intervals during the cuff inflation, and blood flow was calculated between the 2nd and 3rd or between the 3rd and 4th seconds.

Temperatures. Ambient temperature, core temperature adjacent to the carotid artery, and skin temperature on the foot pad and tail adjacent to the laser Doppler probe were measured with 40-gauge copper constantan thermoccuples sampled once every 20 s.

Preparation for experiments. Rats were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal) and gently introduced into a cylindrical plexiglas rat restrainer. The restrainer allowed free access to the hind-leg and tail and to the end of the cannula located between the shoulders. The cannula patency was

tested with physiological saline. Thermocouples were attached to the hind foot pad and mid-way down the tail and inserted into the reentrant tube for monitoring core temperature. Ambient temperature was measured inside the restrainer. Laser Doppler flow probes were positioned on the hind foot pad and tail. The tail was equipped with a pneumatic cuff at its base and the mercury-filled silastic strain gauge, 2 cm distal to the laser Doppler probe. Rats rested undisturbed for 15-30 minutes before injection of saline control or vasoactive substances. The plethysmograph and strain gauge were calibrated during this time. Injections were made either free hand into the cannula with a Hamilton microliter syringe or via a microinfusion pump (Harvard Apparatus, Inc.; Pump model 22).

Protocol. Since norepinephrine (NE) produces a well documented and reliable vasoconstriction in peripheral vasculature, it was selected for a physiological test of our apparatus, instrumentation, and measurements. Fully instrumented rats rested in the restrainer for 15-30 minutes before the intravenous infusion of either 50 μg/kg NE or saline control. All injections were 300 μl in volume. Baseline measurements were begun 5 min preceding the injection, and measurements were continued for at least 35 min post-injection. Experiments were done at air temperatures of 23-27°C.

RESULTS

The effect of saline control and 50 μg/kg NE on blood flow in the foot pad and tail are shown in Figs. 2 and 3. Baseline

values of total tail blood flow (Figs. 2, 3a) and superficial cutaneous microvascular flow in the tail (Figs. 2, 3a) ranged from 2-20 ml/100 ml tissue/min. Microvascular blood flow in the foot pad was normally much higher than in the tail and ranged from 20 to 100 ml/100 ml tissue/min. There was no change in blood flow in response to the saline injection (Fig. 2). Following injection of 50 μg/kg NE, blood flow dropped precipitously at all sites being measured. Since the magnitude of baseline blood flow at the three sites was significantly different (Fig. 3a), values had to be standardized for comparison. Therefore, the effect of NE was assessed by expressing blood flow during the entire experiment as a percentage of baseline values (Figs. 3b and 3c). Body temperatures did not change during the study.

DISCUSSION

The methods and instrumentation described provide a whole animal model for *in vivo* studies of the effects of environmental (thermal) stress and vasoactive substances (exogenous or endogenous) on peripheral blood flow. The baseline values of total tail blood flow (2-100 ml/100 ml tissue/min) are in the range of those reported previously by Raman et al. (7) and Rand et al. (9). The baseline values of superficial cutaneous microvascular flow in the tail agree well with those reported by Thomas et al. (3) and, as expected, are much lower than that observed in the foot pad.

The effectiveness of this model for assessing the impact of vasoactive substances is clearly documented by comparison of the responses to physiological saline used as a control and NE, a known peripheral vasoconstrictor. Whereas physiological saline had no affect on blood flow at any site in response to 50 μl NE, there were comparable precipitous reductions of 70-80% at all three sites monitored. Blood flow expressed as a percentage of baseline (Figs. 3b, c) for comparison of responses demonstrate the uniqueness of each measure and site. For example, while total tail blood flow and tail skin blood flow were reduced by more than 80%, both returned to baseline levels within 6 min. Although foot pad blood flow showed a reduction of similar magnitude, it never returned to baseline during the experiment. Also, while superficial cutaneous microvascular tail blood flow seemed to follow the pattern of total tail blood flow in the first 12 min following administration of NE, it subsequently declined and followed a pattern more similar to that observed in the foot pad. This clearly demonstrates that, although there are sometimes correlations in the two measures, tail blood flow cannot be described by either measure alone. In summary, the model described allows for assessment of the effects of vasoactive substances at different levels in the vascular tree. Furthermore, it allows assessment of the relative homogeneity of responses in different and distinct vascular beds.

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FIGURE LEGENDS

Figure 1. Schematic diagram depicting the whole animal (rat) model for *in vivo* studies of peripheral blood flow. The rat is in a tubular plexiglas restrainer and equipped with thermocouples for measuring skin and core temperatures, laser Doppler flow probes for monitoring superficial cutaneous microvascular blood flow on the tail and foot pad, and a venous occlusion cuff and mercury-filled silastic strain gauge on the tail for monitoring total blood flow in the tail. Rats are surgically equipped with a catheter in the jugular vein or vena cava, and a blind-ended reentrant tube sutured adjacent to the carotid artery. All instrumentation and data collection are controlled by an AT compatible computer, Keithley 575 Measurement and Control System (A/D converter), and LabTech NoteBook software.

Figure 2. Blood flow measurement before (baseline) and after the administration of 300 μl volume of physiological saline as control. No change in blood flow at any site was observed.

Figure 3. Blood flow measurements before (baseline) and after the administration of 50 μg/kg NE in 300 μl volume (between filled triangles at mins 5-6). Top (a) are absolute values; middle (b) are skin

blood flow expressed as percent of baseline flow; bottom (c) is total tail blood flow expressed a percent of baseline.

FIGURE 1

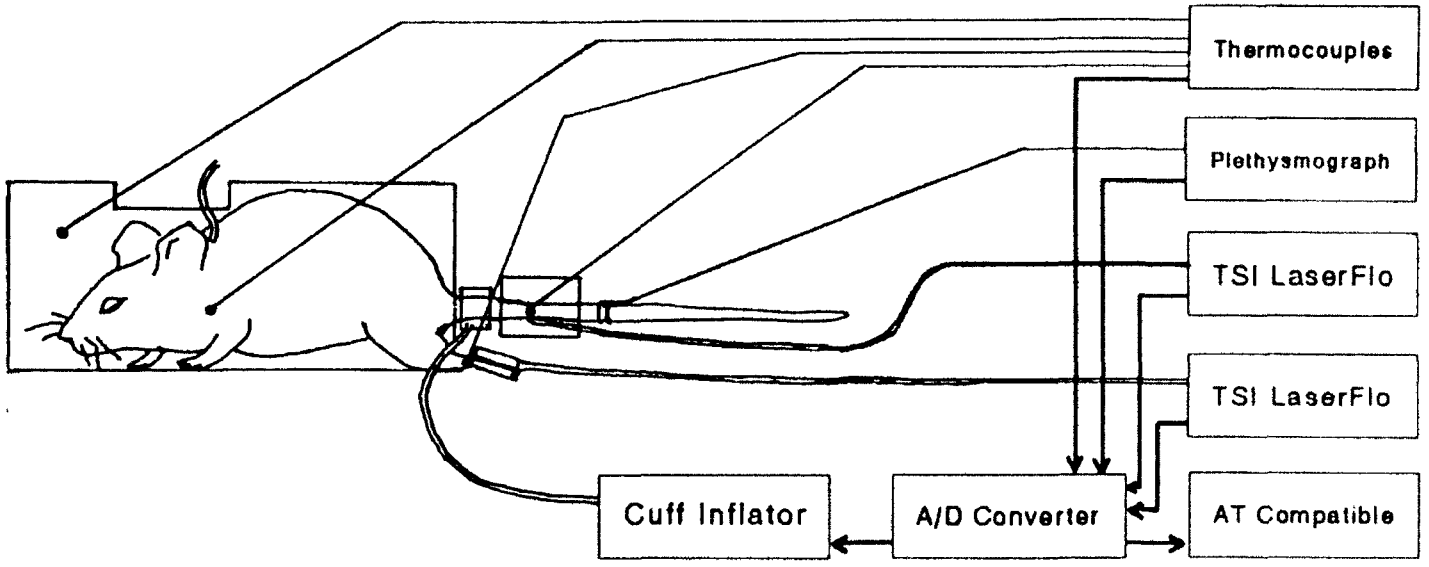

Thermocouples
Plethysmograph
TSI LaserFlo
TSI LaserFlo
Cuff Inflator
A/D Converter
AT Compatible

FIGURE 2

Tail & Foot Blood Flow - absolute values

Saline, 300 µl injection

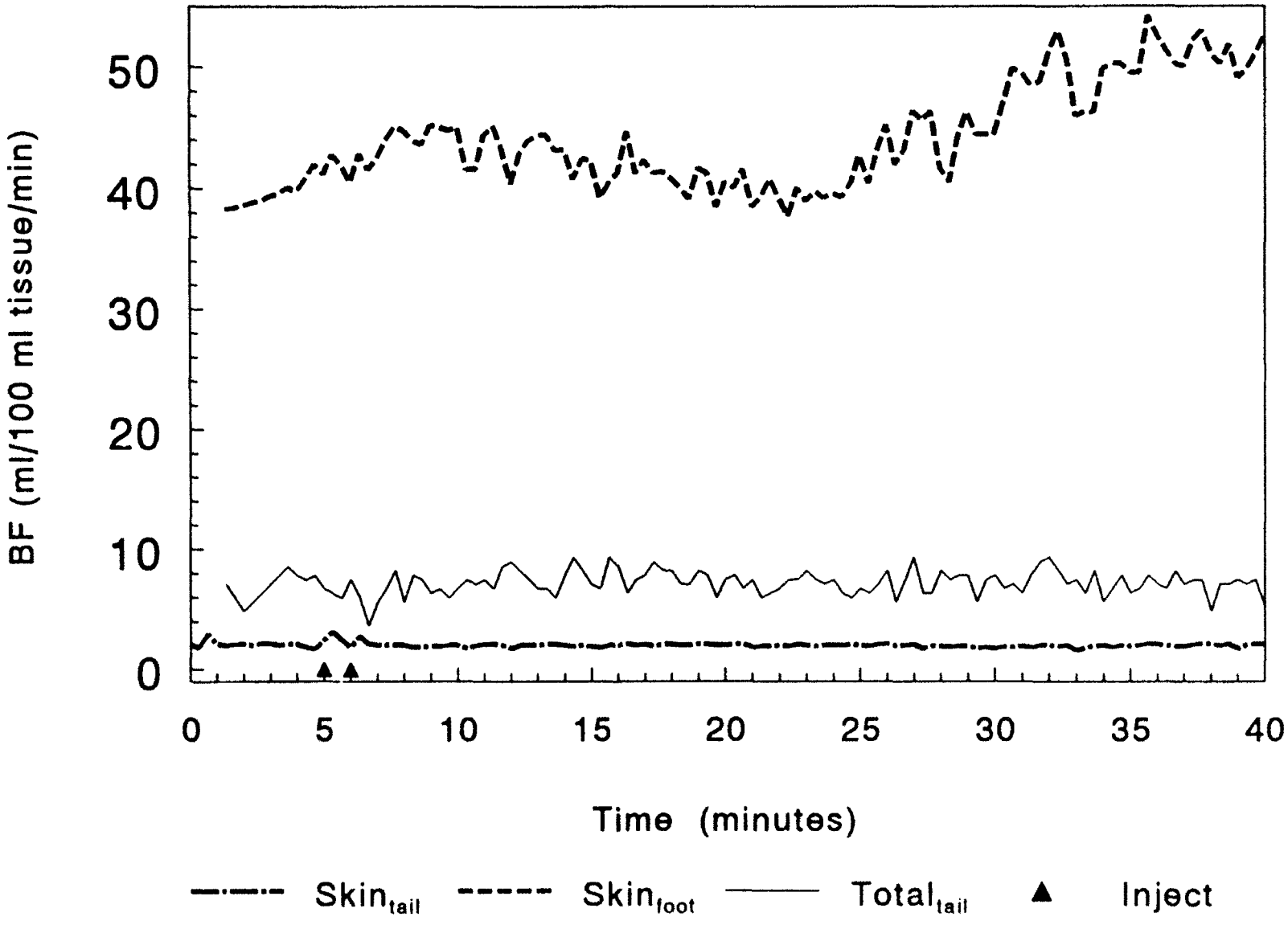
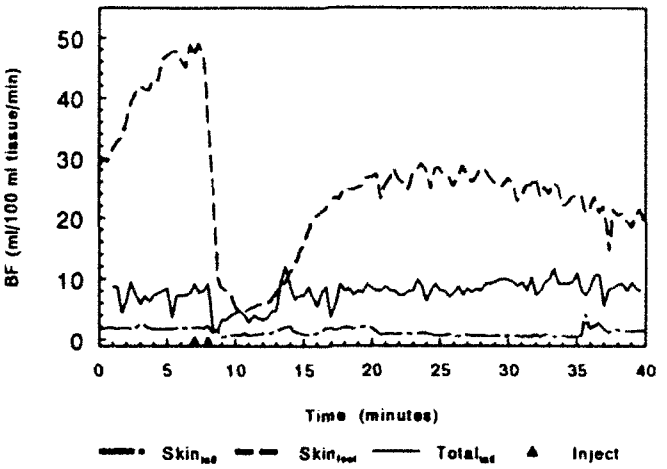


FIGURE 3

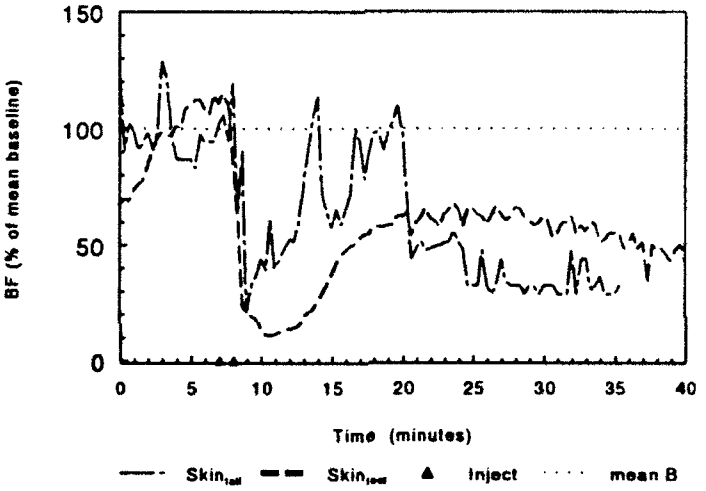
Tail & Foot Blood Flow - absolute values

50 µg/kg Norepinephrine (NE)



Skin Blood Flow - % baseline

50 µg/kg Norepinephrine (NE)



Total Tail Blood Flow - % baseline

50 µg/kg Norepinephrine (NE)